Exhibit A

Method of Imaging Cancer Cells In Vivo Based on Derepression of E2F
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Overview: This invention relates to a method for non-invasive imaging of cancer cells in living animals based on derepression of E2F. For example, this invention could be used to generate mice wherein cancer cells would emit a light signal that could be monitored with a sensitive photon counting camera such as made by Xenogen, Inc.. Such mice could then be used, for example, to test drugs to prevent or treat cancer. By way of another example, this invention could be used to make viruses that give rise to a signal, such as a light signal, when they encounter a cancer cell. Such a virus could be administered to a patient, either locally or systemically, after which cancer cells could be detected through the emission of light.

Background. There are 6 members of the E2F cell-cycle regulatory transcription factor family (E2F1 through E2F6) [1] [2]. These proteins bind to DNA as heterodimers with either DP1 or DP2. 'E2F' is often used as a generic term when describing all 6 family members or the various heterodimers that they are capable of forming. It has been known since the 1980's that many cell-cycle regulated genes contain E2F binding sites including, notably, many genes required for DNA replication specifically and S-phase entry/traversal generally. Work done in the early 1990's showed that E2F is negatively regulated by members of the pRB (retinoblastoma protein) family. Thus, it appeared that free E2F could serve as a transcriptional activator and was silenced upon binding to pRB (or its paralogs). Binding of pRB to E2F is regulated by cell-cycle dependent pRB phosphorylation. Data that began accumulating in the late 1980's indicated that most human tumors harbor mutations that directly or indirectly (that is, through untimely phosphorylation) inactivate pRB [3]. In sum, it has been known for many years that E2F-responsive promoters are cell-cycle regulated and that this is due, at least partly, to cell-the cycle dependent interaction of pRB with E2F.

In the mid 1990s it started to become clear that pRB, rather than passively sequestering E2F, converted E2F from an activator to a potent transcriptional repressor. Thus, elimination of E2F sites in various E2F-responsive promoters was shown to increase, rather than decrease, transcription- presumably due to loss of pRB/E2F transcriptional repressor complexes. PRB preferentially binds to E2F1, E2F2, E2F3, and E2F4. E2F1 through E2F3 are potent activators when not bound to pRB whereas E2F4 is not. Furthermore, E2F1, E2F2, and E2F3 are themselves encoded by E2F-responsive promoters.

The E2F1 promoter has been well studied. It contains 4 canonical E2F binding sites and can render the transcription of a heterologous reporter gene cell-cycle dependent in cell culture experiments [4, 5] [6]. In 1997 Paar et al described a replication-defective adenoviral vector in which a reporter gene (LacZ) was place under the control of a genomic fragment containing the E2F1 promoter [7]. Following infection of C6 glioblastoma cells cell-cycle dependent transcription of LacZ was observed, in keeping with the above. In the next set of experiments, the virus was sterotactically injected into the brains of normal rats or into rats bearing C6 glioblastomas. Robust LacZ expression, as determined by betagalactosidase accumulation, was noted in the tumor tissue but not

surrounding normal brain (in contrast to a control virus in which LacZ was under the control of the cytomegalovirus promoter). Importantly, and not predictable from the cell-culture experiments, the E2F-driven virus did not give rise to detectable LacZ expression when next introduced into rapidly dividing normal cells such as regenerating liver cells following partial hepatectomy. Thus, the E2F-responsive virus appeared to discriminate between cycling normal cells and cycling tumor cells. This discrimination depended upon the integrity of the E2F binding sites in the E2F1 promoter since a virus containing an E2F1 promoter lacking E2F sites now gave rise to LacZ expression in normal brain and diminished LacZ staining in the brain tumors. In a separate set of experiments, Johnson and coworkers have shown that adenoviruses in which certain critical early genes are placed under the control of the E2F1 promoter will selectively replicate in cancer cells, again consistent with the idea that E2F is selectively derepressed in cancer cells [8].

Invention: At the heart of this invention would be an E2F responsive promoter driving the expression of a reporter gene that can be non-invasively imaged in an animal (Fig 1).

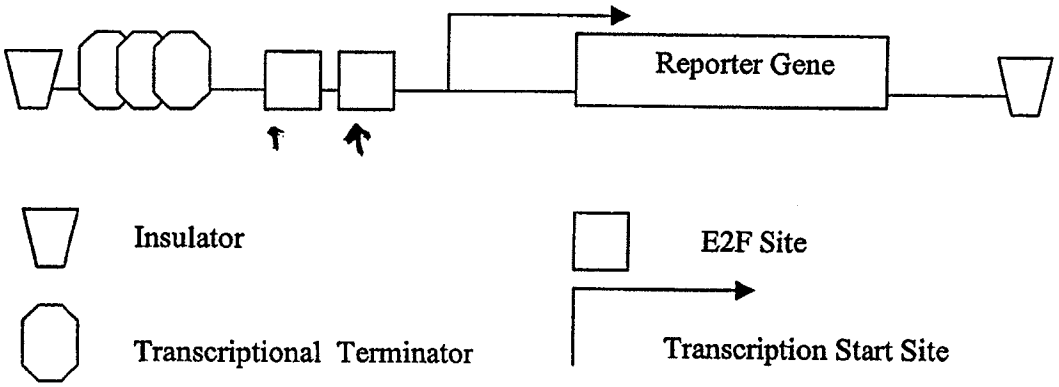

Fig 1. Schematic of E2F-responsive Reporter. For purposes of illustration insulator and transcriptional termination sites have been included.

In one embodiment, the E2F-responsive promoter would be the promoter for E2F1 and the reporter gene would be a firefly luciferase cDNA. The 'E2F1 promoter' is contained within the human genomic sequence 5' of the E2F1 translation start site and contains 4 E2F binding sites (as 2 imperfect palindromes) near the E2F1 transcription start site. In a preferred embodiment the E2F1 promoter fragment would consist of the E2F1 genomic sequences located at –218 to +51 (as used by Parr et al [7]) relative to the translation start site as described by Neuman et al [9]. In another embodiment, the E2F-responsive promoter would be the promoter for p73, which is transcriptionally activated in cancer cells [10]. In yet another embodiment, the E2F-responsive promoter would be a synthetic promoter containing one or more canonical E2F binding sites upstream of a minimal promoter. It will be recognized that the DNA construct containing the E2F responsive

promoter upstream of the reporter gene could be modified further so as to minimize the effects of surrounding DNA sequences following insertion into a DNA based vector and/or integration into a host genome. For example, one or more transcriptional termination sequences could be placed 5' of the E2F-responsive promoter. Likewise, the promoter-reporter construct could be flanked with insulator elements such as those described in [11].

The promoter-reporter construct (hereafter called the 'E2F reporter') could be introduced into animal cells in a variety of ways. Without being limiting, in one embodiment the E2F reporter could be used to make a transgenic animal (such as a mouse) using methods known in the art. For example, a transgenic mouse could be constructed using blastocyst injection or with a lentiviral vector [12]. In another embodiment, the E2F reporter could be made introduced into a genome using homologous recombination (knock-in). In a variation of this approach, a suitable reporter could be knocked-in such that it was placed under the control of an endogenous E2F-responsive promoter. In another embodiment, the E2F reporter could be introduced into a viral or non-viral DNA-based vector system, which could the be used to infect/transduce animal cells in vivo.